Most probable questions from TECHNIQUES IN CELL BIOLOGY | unit 3 Sessional | (Note 4/4)

# Progress (%)	100
Status	In progress
Content Type	Notes
Chapter	CYTOSKELETAL PROTEINS AND TECHNIQUES IN CELL BIOLOGY TECHNIQUES IN CELL BIOLOGY
Course	Graduation
Reference	
Subject	Biochemistry
Topic	Microscope Stain
Remember in your prayers. Last update	@January 30, 2023 11:02 PM

Topics covered here includes:

Visualization of cells and sub-cellular components by light microscopy, different fixation and staining techniques for EM, freeze itch and freeze fracture methods for EM.

❓ What is light microscopy and how is it used to visualize cells and sub-cellular components?
Light microscopy is a type of microscopy that uses visible light and lenses to magnify the images of cells and sub-cellular components. Light microscopy allows for the visualization of cells and organelles in living or fixed and stained samples. Some common staining techniques used for light microscopy include H&E staining, which stains for cellular structures and differentiates cell types, and GFP tagging, which allows for visualization of specific proteins within cells.

❓ What is electron microscopy and how does it differ from light microscopy?
Electron microscopy (EM) is a type of microscopy that uses a beam of electrons to produce high-resolution images of cells and sub-cellular components. EM provides greater resolution and magnifies objects much more than light microscopy, making it ideal for visualizing fine cellular structures such as proteins, ribosomes, and membranes.

❓ What is the role of fixation and staining techniques in electron microscopy?
Fixation and staining techniques play a crucial role in electron microscopy by preserving the structure of cells and sub-cellular components, allowing them to be visualized by the electron beam. Fixation stabilizes the cells and organelles, preventing them from collapsing or changing shape during preparation for EM. Staining is used to enhance the contrast between different cellular structures, making them more visible in the EM image. Some common fixation and staining techniques for EM include glutaraldehyde and osmium tetroxide fixation, and uranyl acetate or lead citrate staining.

❓ What are freeze-etch and freeze-fracture methods in electron microscopy?
Freeze-etch and freeze-fracture methods are techniques used in electron microscopy to visualize the internal structure of cells and sub-cellular components, particularly lipid-containing membranes. These methods involve rapidly freezing the sample and then either etching away the frozen material or physically breaking the frozen material to reveal the internal structure. Freeze-etch and freeze-fracture methods provide high-resolution images of the lipid bilayer and other internal structures, allowing for the study of membrane structure and function.

❓ How does the freeze-etch method work in electron microscopy?
The freeze-etch method involves rapidly freezing the sample, followed by etching the frozen material to reveal the internal structure. The sample is mounted on a metal grid and then exposed to a rapidly evaporating liquid, such as ethanol, which cools the sample rapidly. The frozen material is then etched away with a reactive gas, such as a mixture of oxygen and argon, which removes the frozen material layer by layer, exposing the internal structures. The exposed structures are then visualized with an electron microscope. This method provides high-resolution images of the lipid bilayer and other internal structures, allowing for the study of membrane structure and function.

❓ What are the advantages and disadvantages of light microscopy compared to electron microscopy?
Advantages of light microscopy:

- Non-invasive: Light microscopy allows for the visualization of cells and sub-cellular components in living samples, making it a non-invasive method of analysis.
- Cost-effective: Light microscopy is typically less expensive than electron microscopy due to the lower cost of the equipment and the ease of use.
- Accessible: Light microscopy is widely available in research and medical laboratories and is a well-established technique.

Disadvantages of light microscopy:

- Limited resolution: Light microscopy has a lower resolution compared to electron microscopy, making it unsuitable for the study of fine cellular structures.
- Photodamage: Visualizing cells and sub-cellular components with light microscopy can cause photodamage to the sample, which can affect the outcome of experiments.

Advantages of electron microscopy:

- High resolution: Electron microscopy provides high-resolution images of cells and sub-cellular components, making it ideal for the study of fine cellular structures such as proteins, ribosomes, and membranes.
- Detail: Electron microscopy provides much more detail than light microscopy, allowing for the study of complex cellular structures and processes.

Disadvantages of electron microscopy:

- Invasive: Electron microscopy requires fixation and staining of the sample, which can alter the structure of cells and sub-cellular components, making it an invasive method of analysis.
- Complex: Electron microscopy requires specialized equipment and expertise, making it a more complex and time-consuming technique compared to light microscopy.

❓ What are some common applications of light microscopy and electron microscopy?
Common applications of light microscopy include the study of cell biology, development, and physiology, as well as the visualization of cells and tissues in medical diagnoses.
Common applications of electron microscopy include the study of cellular ultrastructure, the visualization of internal structures of cells and sub-cellular components, and the analysis of materials and structures at the nanoscale.

❓ What is the role of fixation in electron microscopy?
Fixation is an important step in electron microscopy as it stabilizes the sample, preventing it from undergoing any further changes during preparation and imaging. This helps to preserve the native structure of the cells and sub-cellular components, allowing for accurate visualization and analysis. There are many different fixation methods, each with its own strengths and weaknesses, that can be used depending on the sample and the specific application.

❓ What is the role of staining in electron microscopy?
Staining is a crucial step in electron microscopy as it helps to enhance the contrast of cells and sub-cellular components, making them more easily visible in the electron microscope. This is done by using a variety of dyes and stains that selectively target specific structures within the cell, such as proteins, lipids, and carbohydrates. The staining procedure can also help to preserve the native structure of the cells and sub-cellular components during preparation and imaging, allowing for accurate visualization and analysis.

❓ What is the difference between negative staining and positive staining in electron microscopy?

Negative staining involves the application of a negatively charged stain to the sample, which does not penetrate into the cells and sub-cellular components but instead forms a thin layer around them. This helps to enhance the contrast of the cells and sub-cellular components in the electron microscope by creating a clear background.

Positive staining involves the application of a positively charged stain to the sample, which selectively penetrates into specific structures within the cells and sub-cellular components. This helps to highlight specific structures, such as proteins, lipids, and carbohydrates, by staining them with a bright, highly visible dye. Positive staining can be used to study the fine structure and composition of cells and sub-cellular components.

❓ What are freeze-etching and freeze-fracture methods for EM?

Freeze-etching and freeze-fracture methods are techniques used in electron microscopy to study the ultrastructure of cells and sub-cellular components, particularly their internal organization and distribution.

In freeze-etching, the sample is rapidly frozen and then etched with an electron-beam to produce a replica of the internal structure of cells and sub-cellular components. This replica is then examined in the electron microscope, allowing for high-resolution visualization of the internal ultrastructure of cells and sub-cellular components.

Freeze-fracture involves the rapid freezing of the sample and the creation of a fracture plane in the material. The fractured surface is then replicated and examined in the electron microscope, allowing for the study of the internal organization and distribution of cells and sub-cellular components, particularly lipids and membranes.

These techniques are particularly useful for the study of biological membranes, as they allow for the visualization of the lipid bilayer and the associated proteins. They are also valuable for the study of cellular ultrastructure, as they provide detailed information about the internal organization and distribution of cells and sub-cellular components.

❓ What are some benefits and limitations of the freeze-etching and freeze-fracture methods?
Benefits:

- High-resolution: Freeze-etching and freeze-fracture methods provide high-resolution images of cells and sub-cellular components, making them ideal for the study of fine cellular structures such as proteins, ribosomes, and membranes.
- Internal structures: These methods allow for the visualization of the internal ultrastructure of cells and sub-cellular components, providing valuable information about their organization and distribution.
- Membranes: Freeze-etching and freeze-fracture methods are particularly useful for the study of biological membranes, allowing for the visualization of the lipid bilayer and the associated proteins.
  Limitations:
- Technical difficulty: Freeze-etching and freeze-fracture methods are complex techniques that require specialized equipment and expertise, making them more challenging and time-consuming compared to other methods.
- Invasive: These methods require the fixation and staining of the sample, which can alter the structure of cells and sub-cellular components, making them invasive methods of analysis.
  Photodamage: Freeze-etching and freeze-fracture methods can cause photodamage to the sample, which can affect the outcome of experiments.

❓ What is electron microscopy (EM)?
Electron microscopy (EM) is a technique used to visualize cells and sub-cellular components at high resolution. This technique uses a beam of electrons to produce images of the sample, providing detailed information about the ultrastructure of cells and sub-cellular components.

❓ What are some benefits and limitations of electron microscopy?
Benefits:

- High-resolution: Electron microscopy provides high-resolution images of cells and sub-cellular components, allowing for the study of fine cellular structures such as proteins, ribosomes, and membranes.
- Internal structures: Electron microscopy allows for the visualization of the internal ultrastructure of cells and sub-cellular components, providing valuable information about their organization and distribution.
- Membranes: Electron microscopy is particularly useful for the study of biological membranes, allowing for the visualization of the lipid bilayer and the associated proteins.

Limitations:

- Sample preparation: Electron microscopy requires the fixation and staining of the sample, which can alter the structure of cells and sub-cellular components.
- Technical difficulty: Electron microscopy is a complex technique that requires specialized equipment and expertise, making it a challenging and time-consuming method of analysis.
- Invasive: Electron microscopy is an invasive method of analysis, as the sample preparation process can affect the structure of cells and sub-cellular components.
- Sample size: Electron microscopy is limited by the size of the sample that can be analyzed, as larger samples cannot be imaged in their entirety.

❓ What is fixation in electron microscopy?
Fixation is a process used in electron microscopy to preserve the structure of cells and sub-cellular components for analysis. This is achieved by treating the sample with a fixative solution, which stabilizes the cellular structures and prevents them from being damaged during the subsequent preparation process.

❓ What are some common fixatives used in electron microscopy?
Common fixatives used in electron microscopy include:

- Formaldehyde
- Glutaraldehyde
- Osmium tetroxide
- Paraformaldehyde

❓ What is staining in electron microscopy?
Staining in electron microscopy is a process used to enhance the contrast of cells and sub-cellular components in images produced by the electron microscope. This is achieved by treating the sample with a stain, which makes specific structures within the cells and sub-cellular components more visible and distinguishable.

> Do you have any notes from ma'am of unit 3❓Please share in group. Topics not covered here will be added in notes. Updated notes will be share in group. All notes will also be share at the time of examination. so stay tuned…
>
> Signing off.
>
> Yours💔….(friend bhai friend)